

## EXHIBIT

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### DEFENDANTS' MOTION TO EXCLUDE THE TESTIMONY OF DR. CHRISTOPHER TEAF

05-CV-0329 GKF-PJC

**IN THE UNITED STATES DISTRICT COURT  
FOR THE NORTHERN DISTRICT OF OKLAHOMA**

**STATE OF OKLAHOMA, ex rel. W.A. DREW  
EDMONDSON, in his capacity as ATTORNEY  
GENERAL OF THE STATE OF OKLAHOMA  
AND OKLAHOMA SECRETARY OF THE  
ENVIRONMENT C. MILES TOLBERT, in his  
capacity as the TRUSTEE FOR NATURAL  
RESOURCES FOR THE STATE OF  
OKLAHOMA**

**PLAINTIFFS**

v.

**CASE NO.: 05-CV-00329 GKF -SAJ**

**TYSON FOODS, INC., TYSON POULTRY, INC.,  
TYSON CHICKEN, INC., COBB-VANTRESS,  
INC., CAL-MAINE FOODS, INC., CAL-MAINE  
FARMS, INC. CARGILL, INC., CARGILL  
TURKEY PRODUCTION, LLC, GEORGE'S,  
INC., GEORGE'S FARMS, INC., PETERSON  
FARMS, INC., SIMMONS FOODS, INC. and  
WILLOW BROOK FOODS, INC.**

**DEFENDANTS**

**Report by Dr. Samuel Myoda**

reflects that the mean bacteria concentration for *E. coli* is 4,174 CFU/100ml, for enterococcus is 14,664 CFU/100ml, and for faecal coliforms is 6,371 CFU/100ml. Although there were a few samples reported to have concentrations of 1,600,000 CFU/100ml, those are atypical and represent outliers in the data set. However, even those outlying values are an order of magnitude below that of sewage influent (5,400,000 CFU *E. coli*/100ml, Miyanaga et. al, 2006, *Detection of Escherichia coli in the sewage influent by fluorescent labeled T4 phage*). In my view, the atypically high values are more consistent with samples taken in close proximity to a concentrated source of indicator bacteria, e.g. cattle feces, than with runoff samples taken from areas affected by uniformly distributed indicator bacteria such as the application of poultry litter.

7.3. The indicator bacteria in the waters of the IRW originate from many sources. The loading from cattle is extremely significant. Typically, cattle will excrete 15 to 35 kg of feces per day. In the summer when the majority of primary contact recreation is occurring, the initial *E. coli* concentration in the cattle feces will be approximately 3,000,000 CFU *E. coli*/gram. However, after deposition the bacteria multiply and reach levels up to 48,000,000 CFU *E. coli*/gram (Sinton et. al, 2007, *Survival of Indicator and Pathogenic Bacteria in Bovine Feces on Pasture*). Also in the summer months the cattle tend to congregate near and in the streams in order to cool off, increasing the possibility of direct deposition into and in close proximity of the streams. This means that each day one cow will contribute roughly 960,000,000,000 *E. coli* into the environment and with approximately 200,000 head of cattle in the IRW over 192,000,000,000,000 CFU *E. coli* will be introduced into the environment each day. Contrasting the growth of indicator bacteria in a “cow pie” is the death of indicator bacteria in the litter via composting in the poultry houses and by direct exposure to UV radiation. The litter is spread in a very thin layer on the surface of a field, it is dry and the indicator bacteria

are not protected as they are in "cow pies". Dr. Harwood testified that she believed that the indicator bacteria in poultry litter would only survive for a few hours after application.

7.4. In addition to cattle, there are approximately 150,000 swine and numerous wildlife (geese, ducks, deer, turkeys, other birds, small mammals, rodents, etc.) that live throughout or migrate through the watershed. The wastewater treatment plant effluent and septic system loads are additional sources of indicator bacteria. As indicated in the numerous TMDLs that have been established in OK, wildlife are significant sources of fecal material and indicator bacteria. The USGS reported that in Delaware County, Oklahoma, 45% of the *E. coli* sampled came from birds and 22% came from cattle (*Reconnaissance of the Hydrology, Water Quality, and Sources of Bacterial and Nutrient Contamination in the Ozark Plateaus Aquifer System and Cave Springs Branch of Honey Creek, Delaware County, Oklahoma, March 1999–March 2000*).

## **8. Pathogenic Bacteria Not Detected in the IRW**

8.1. The presence of indicator bacteria does not mean that pathogens are present. The pathogens that the Plaintiffs claim to be present include salmonella, campylobacter and *E. coli* O157 (a strain of *E. coli* that is pathogenic to humans). These pathogens are carried by a variety of hosts. For example, *E. coli* O157 are primarily found in cattle, salmonella in reptiles and poultry, and campylobacter in cattle, swine and poultry. Other hosts could carry these pathogens as well. The Plaintiffs contend that *E. coli* O157 is shed from poultry. However, there is virtually no evidence that poultry carries *E. coli* O157 and the Plaintiffs never tested for or found it in the litter or in the environment. Campylobacter does not survive well in the environment. It will die when exposed to oxygen and will also readily dehydrate and die. We find the Plaintiffs' campylobacter testing results in the IRW not surprising. No campylobacter was found in litter, ground water, dust, soil, public water supply or spring water samples and

only 2 out of 302 surface water samples contained campylobacter. We find the Plaintiffs' salmonella testing results in the IRW not surprising as well. No salmonella was found in ground water, dust, soil, public water supply or spring water samples and only 24 out of 562 surface water samples and 2 out of 17 litter samples contained salmonella. In both the salmonella and campylobacter positive samples, the source(s) were not identified.

The basic precept in the field of regulatory control of the microbiological status of water and environmental samples is the identification and enumeration of viable indicator organisms and pathogens as the only predictor of the public health status of a specific material. A wide spectrum of specific tests and criteria has been developed to grow and enumerate microorganisms in the laboratory setting. The specifics and usages of these analyses are listed in the Federal Register and have been published in the Standard Methods for the Examination of Water and Wastewater. Using the results from these testing procedures, US EPA and state and local regulatory entities enforce the standards set up by the appropriate governing bodies. It is only through the use of such scientifically sound and verifiable testing criteria that effective enforcement can be accomplished for the public's benefit.

The concept that some potentially pathogenic microorganisms may be present in the environment but are in a metabolic state from which they cannot be grown in the laboratory (viable but not culturable or VBNC) has been reported. How a situation concerning VBNC organisms in the environment may impact human health is a matter of dispute. In some studies, the appropriate application of suitable culturing conditions have been questioned, cells subsequently observed were from a very minor subpopulation viable cells (2, 4, 9, 11) or cells adapted to utilize the nutrients released from dead cells (3) in the original sample. Some studies have used tests and assays that measure both live and dead cells, never reflecting on the fact that the dead cells posed no threat to humans. Resuscitating VBNC organisms often

takes non-physiological conditions, referred to by one researcher as “exquisite laboratory conditions” (8). Winfield and Groisman state, “Recovery from a VBNC state occurs rarely, if at all” (17). Studies using VBNC cells directly indicated that pathogenicity was not seen (4, 5, 6, 8, 10, 13, 14, 15, 17). In fact some researchers consider the concept of ‘viable but not culturable’ as an oxymoron (1). Barer and Bogosian (2004) summarize the metabolic status of VBNC cells, stating, “that the observed nonculturable cells are either dead or passing through a brief injured state to death.” For effective regulation and enforcement in monitoring environmental samples for potential health threats, there is a necessity for verifiable identification of microorganisms in a metabolic state and in sufficient numbers to initiate a disease process in humans. No evidence exists to support such conditions arising from VBNC organisms in the environment. The microbiological regulatory field is based on the enumeration of viable organisms and not on detecting the presence of material that does not pose a threat to human health. Plausible predictions on the safety of food, potable water, and wastewater effluent would be impossible without reliable and reproducible assays to quantify the actual levels of viable suspect organisms in these materials.

The Plaintiffs’ explain that the negative results of their tests for pathogenic salmonella and campylobacter are due to the fact that these organisms are present but in the VBNC state. This is not true. The Plaintiffs’ did not find them because they were not present. The lack of these pathogens and confirmation of their negative results could have easily been confirmed using molecular methods. In fact, e-mail correspondence and lab a notebook entry indicates that some samples were tested for pathogens using PCR. However, we were unable to find any of those results in the material that the Plaintiffs’ provided and Dr. Harwood indicated in her deposition that she did not use PCR to test for pathogens. We find it very puzzling that the written records do no match her testimony. In addition, in support of the Plaintiffs’ VBNC

theory, Dr. Harwood's report states that "Many studies have indicated that pathogens which enter the VBNC state remain infectious (Baffone et al., 2003; Oliver and Bockian, 1995) including Campylobacter jejuni (Baffone et al., 2006) and E. coli O157:H7 (Makino et al, 2000)." This is very misleading. Baffone et al., 2003 reports that cells from 3 strains of Vibrio were incubated in salt water until their viable counts were >0.1cfu/ml. 0.1 ml was used to infect mice per gastric. Subsequently mice were sacrificed and the gut tissue cultured. Recoveries of the inoculated Vibrio strain were seen in 25-50% of the mice. It took 2 sequential mouse passages for the organisms to recover pathogenic characteristics. Vibrio is not found in chickens, it is a pathogen of concern in shellfish. Baffone et al., 2006 reported that using similar inoculation criteria with different strains of campylobacter, it was isolated from less than 30% of mice tested. None were deemed 'infected' as was stated in the Harwood report – rather we can only say that they were 'resuscitated'. Furthermore,  $\sim 10^4$  metabolically active cells (though 'nonculturable') were needed to effect subsequent recovery of campylobacter from the mouse gut. This number is significantly higher than what is needed for normal metabolically active cells and is significantly higher than concentrations typically found in the environment.

## 9. Plaintiffs' Flawed "Biomarker"

9.1. Plaintiffs purport to have identified a poultry specific "biomarker", which they allege allows them to specifically identify bacterial contamination derived from poultry sources. Plaintiffs further assert that their process allows them to quantify the amount of "biomarker" which they suggest correlates with the presence of indicator bacteria. The development of the Plaintiffs' "biomarker" process was deeply flawed in many ways. In fact, their conclusion that their "biomarker" is specific to poultry is demonstrably false.